

artus[®] Malaria RG PCR Kit

Handbook



24 (catalog no. 4601203)

For research use only. Not for use in diagnostic procedures.

For use with the *artus 3000*[™] and the *Rotor-Gene*[™] 3000

June 2007 – Version 1



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artus Malaria RG PCR Kit

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artus[®] Malaria RG PCR Kit

For use with the *artus 3000*[™] or the *Rotor-Gene*[™] 3000^{*}.

For research use only. Not for use in diagnostic procedures.

1. Contents

	Labelling and contents	Art. No. 4601203 24 reactions
Blue	<i>Malaria RG Master</i>	2 x 12 rxns
Red	<i>Malaria RG QS 1[¤]</i> <i>7 x 10⁴ cop/μl</i>	1 x 200 μl
Red	<i>Malaria RG QS 2[¤]</i> <i>7 x 10³ cop/μl</i>	1 x 200 μl
Red	<i>Malaria RG QS 3[¤]</i> <i>7 x 10² cop/μl</i>	1 x 200 μl
Red	<i>Malaria RG QS 4[¤]</i> <i>7 x 10¹ cop/μl</i>	1 x 200 μl
Green	<i>Malaria RG IC[¤]</i>	1 x 1,000 μl
White	<i>Water (PCR grade)</i>	1 x 1,000 μl

[¤] QS = Quantitation Standard
IC = Internal Control

2. Storage

The components of the *artus* Malaria RG PCR Kit should be stored at -20°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at +4°C should not exceed a period of five hours.

^{*} The *artus* Malaria RG PCR Kit can be used with the *Rotor-Gene*[™] 2000 as well.

3. Additionally Required Materials and Devices

- Disposable powder-free gloves
- DNA isolation kit (see **8.2 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *artus 3000™* or *Rotor-Gene™ 3000*
- 0.1 ml PCR tubes for use with 72-well rotor (0.1 ml Strip Tubes and Caps, QIAGEN Hamburg, Cat. No.: 4699982; 0.1 ml tubes, Corbett Research, Cat. No.: ST-1001)
- Alternatively: 0.2 ml PCR tubes for use with 36-well rotor (e.g. 0.2 ml PCR Tubes, QIAGEN Hamburg, Cat. No.: 4699983; 0.2 ml tubes, Corbett Research, Cat. No.: SE-1003F)
- Cooling Block (72-/96-Well Loading Block, QIAGEN Hamburg, Cat. No.: 4699980/ 4699981; 72/96 well loading block, Corbett Research, Cat. No.: 3001-008/3001-009)

4. General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the Cooling Block (72/96 well loading block).

5. Pathogen Information

Malarial infections occur mostly in the tropics and subtropics. Four different human pathogens can be distinguished that cause three malarial diseases of differing severity. Malaria tropica, which follows an infection with *Plasmodium falciparum*, is the most dangerous of the three disease forms and can lead to death within days of infection. Malaria is usually transmitted via bites from the *Anopheles* mosquito, but also via blood transfusions, hypodermic needle sharing among intravenous drug users (IVDU), transplantations and, only rarely, prenatally. Following an initial attack of the liver, the pathogens reach the blood stream. After infiltration of the plasmodia into erythrocytes, they commence proliferation. Eventually the erythrocytes burst and the released pathogens are free to attack further erythrocytes. This typical cyclic course of the disease leads to general symptoms such as headaches, lassitude, nausea and fever, which may appear in recurrent episodes with malaria tertiana and malaria quartana.

The *artus* Malaria RG PCR Kit provides a fast determination from EDTA-blood of all *Plasmodium* species affecting humans.

The four species of *sporozoans* infect humans with the following diseases:

Pathogen	Disease
<i>Plasmodium falciparum</i>	Malaria tropica
<i>Plasmodium vivax</i>	Malaria tertiana
<i>Plasmodium ovale</i>	Malaria tertiana
<i>Plasmodium malariae</i>	Malaria quartana

6. Principle of Real-Time PCR

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e. in real-time)

allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run (Mackay, 2004).

7. Product Description

The *artus* Malaria RG PCR Kit constitutes a ready-to-use system for the detection of *Plasmodium* DNA using polymerase chain reaction (PCR) in the *artus 3000™* or *Rotor-Gene™ 3000*. The *Malaria RG Master* contains reagents and enzymes for the specific amplification of a 163 bp region of the *Plasmodium* genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling A.FAM of the *artus 3000™* or *Rotor-Gene™ 3000*. In addition, the *artus* Malaria RG PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control (IC)* in fluorescence channel Cycling A.JOE. The detection limit of the analytical *Plasmodium* PCR (see **11.1 Analytical Sensitivity**) is not reduced. External positive controls (*Malaria RG QS 1 - 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **8.4 Quantitation**.

8. Protocol

8.1 Pre-analytics: Specimen Collection, Storage and Transport

Attention: Current studies refer to EDTA blood as the most suitable sample material. Therefore, we recommend the use of this material with the *artus* Malaria RG PCR Kit.

The internal validation of the *artus* Malaria RG PCR Kit has been performed using EDTA samples. Other sample materials are not validated. Please use only recommended nucleic acid isolation kits (see **8.2 DNA Isolation**) for sample preparation.

Following instructions regarding collection, transport and storage have to be strictly observed.

8.1.1 Specimen Collection

The use of commercially available anti-agglutination tubes for blood sampling is essential. After blood letting tubes must be shaken carefully in order to prevent clogging. Errors during blood sampling may lead to false negative results. Taking blood between fever attacks may lead to notably smaller numbers of parasitaemia due to small sample volumes in the PCR (5 µl out of 200 µl blood sample). Therefore, blood letting must be done during a fever attack. Furthermore, fast aspiration as well as prolonged puncture time bear the risk of hemolysis and may lead to coagulation of blood samples.

8.1.2 Sample Storage

The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.

To store the samples for a short time (< 2 days) room temperature is ideal. If the specimens are not processed within two days, they have to be stored at +4 °C. If cooling is not possible or if samples have to be stored for longer time, we recommend to use the FTA Card System (Whatman). If specimens are sent to a diagnostic laboratory, the samples have to be transported as soon as possible and must be processed within one day.

8.1.3 Sample Transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection or loss of sample due to a leakage of sample (e.g. cullet) can be avoided. If this occurs, the samples have to be collected again.

If blood specimens are sent to a diagnostic laboratory, transport and sample procession must occur within two days at room temperature. The samples

should be transported following also the local and national instructions for the transport of pathogen material.*

8.2 DNA Isolation

Various manufacturers offer DNA isolation kits. Sample amounts for the DNA isolation procedure depend on the protocol used. Please carry out the DNA isolation according to the manufacturer's instructions. The following isolation kit is recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer
EDTA blood	High Pure PCR Template Preparation Kit	1 796 828	Roche Diagnostics

- **Carrier RNA** is not required.
- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* Malaria RG PCR Kit.
- Blood collection tubes coated with **anticoagulants** may inhibit the PCR. However, these inhibitors will be eliminated by the use of the isolation kits listed above. It is recommended to avoid the use of heparin blood.
- When using isolation protocols with **ethanol**-containing washing buffers, please carry out an additional centrifugation step (three minutes, 13,000 rpm) before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
- The *artus* Malaria RG PCR Kit should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* Malaria RG PCR Kit can be used directly in the isolation procedure (see **8.3 Internal Control**).

* International Air Transport Association. Dangerous Goods Regulations, 41st Edition, 2000.704.

8.3 Internal Control

An *Internal Control (Malaria RG IC)* is supplied. This allows the user **both to control the DNA isolation procedure and to check for possible PCR inhibition** (see Fig. 1). For this application, add the *Internal Control* to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the High Pure PCR Template Preparation Kit (Roche Diagnostics) the DNA is eluted in 50 µl elution buffer. Hence, 5 µl of the *Internal Control* should be added **after** the Proteinase K treatment step (step 1 of the High Pure PCR Template Preparation Kit). The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* should be added only

- to the mixture of lysis buffer, sample material and Proteinase K or
- directly to the lysis buffer.

The *Internal Control* must not be added to the sample material directly. If added to the lysis buffer please note that the mixture of *Internal Control* and lysis buffer has to be prepared freshly and used instantly (storage of the mixture at room temperature or in the fridge for only a few hours may lead to *Internal Control* failure and a reduced extraction efficiency). Please do **not** add the *Internal Control* to the sample material directly.

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 0.5 µl of the *Internal Control* per reaction directly to 15 µl *Malaria RG Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above^{*} and add 5 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *Malaria RG Master* and the *Internal Control* according to the number of samples (see **8.5 Preparing the PCR**).

^{*} The volume increase caused by adding the *Internal Control* is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

8.4 Quantitation

The enclosed *Quantitation Standards (Malaria RG QS 1 - 4)* are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the *artus 3000™* or *Rotor-Gene™ 3000*, all four *Quantitation Standards* should be used and defined in the menu window *Edit Samples* as standards with the specified concentrations (see *artus 3000™ Software Manual* or *Rotor-Gene™ Manual, Version 4.6*). The standard curve generated as above can also be used for subsequent runs, provided that at least one standard of **one** given concentration is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *artus 3000™ Software Manual* or *Rotor-Gene™ Manual, Version 4.6*). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

Attention: The *Quantitation Standards* are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/}\mu\text{l)} \times \text{Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

Please note that as a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g. narrowing the volume by centrifugation or increase of volume by replenishment to the volume required for the isolation).

Important: A guideline for the quantitative analysis of *artus* systems on the *artus 3000™* or *Rotor-Gene™ 3000* is provided at www.qiagen.com/Products/ByLabFocus/MDX (**Technical Note for quantitation on the *artus 3000™* or *Rotor-Gene™ 3000***).

8.5 Preparing the PCR

Make sure that the Cooling Block (accessory of the *artus 3000™* or *Rotor-Gene™ 3000*) is pre-cooled to +4°C. Place the desired number of PCR tubes into the Cooling Block. Please make sure that at least one *Quantitation Standard* as well as one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards (Malaria RG QS 1 - 4)* for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control to monitor the DNA isolation procedure and to check for possible PCR inhibition*, it has already been added to the isolation (see **8.3 Internal Control**). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

	Number of samples	1	12
1. Preparation of Master Mix	<i>Malaria RG Master</i>	15 µl	180 µl
	<i>Malaria RG IC</i>	0 µl	0 µl
	Total Volume	15 µl	180 µl
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total Volume	20 µl	20 µl each

If you want to use the *Internal Control exclusively to check for PCR inhibition*, it must be added directly to the *Malaria RG Master*. In this case, please use the following pipetting scheme (for a schematic overview see Fig. 2):

	Number of samples	1	12
1. Preparation of Master Mix	<i>Malaria RG Master</i>	15 µl	180 µl
	<i>Malaria RG IC</i>	0.5 µl	6 µl
	Total Volume	15.5 µl*	186 µl*
2. Preparation of PCR assay	Master Mix	15 µl*	15 µl each*
	Sample	5 µl	5 µl each
	Total Volume	20 µl	20 µl each

Pipette 15 µl of the Master Mix into each PCR tube. Then add 5 µl of the eluted sample DNA to each tube and mix well by pipetting up and down several times. Correspondingly, 5 µl of at least one of the *Quantitation Standards (Malaria RG QS 1 - 4)* must be used as a positive control and 5 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes. Please take care that the *Locking Ring* (accessory of the *artus 3000™* or *Rotor-Gene™ 3000*) is placed on top of the rotor to prevent accidental opening of the tubes during the run.

* The volume increase caused by adding the *Internal Control* is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Addition of the *Internal Control* to the Purification Procedure

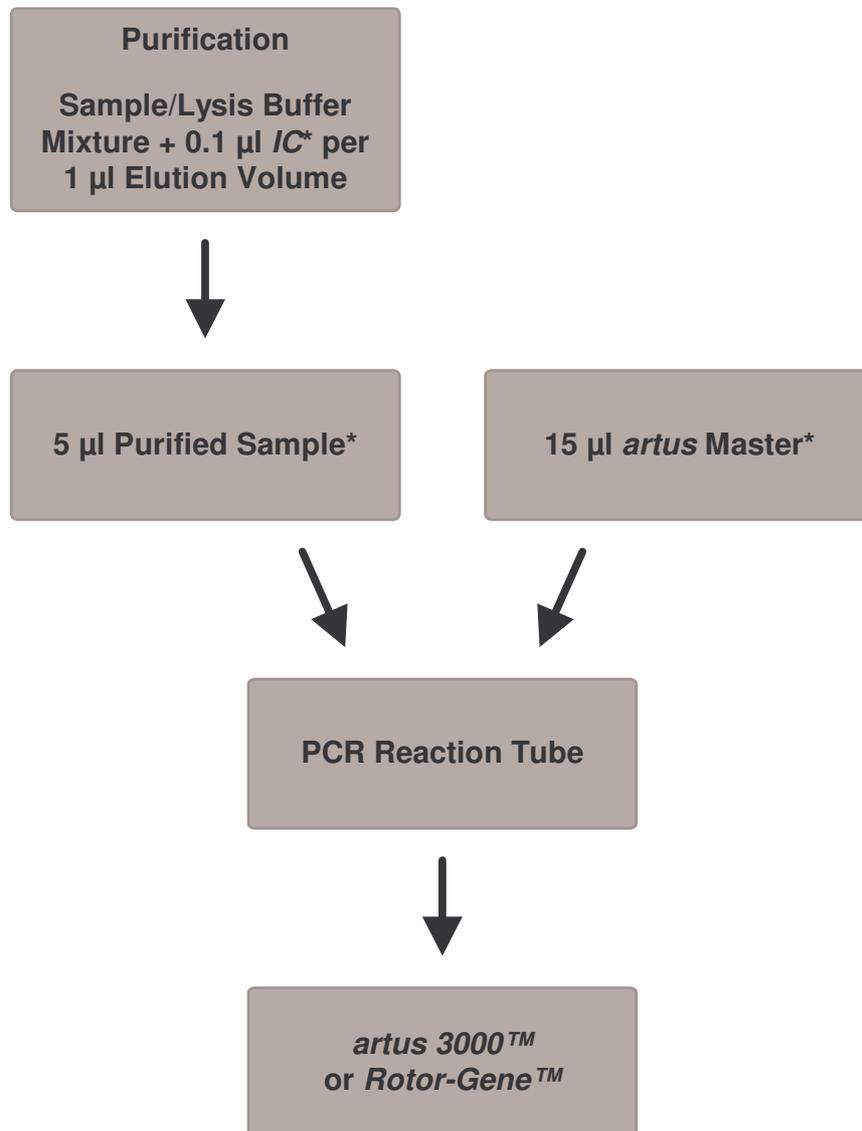


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

Addition of the *Internal Control* into the *artus* Master

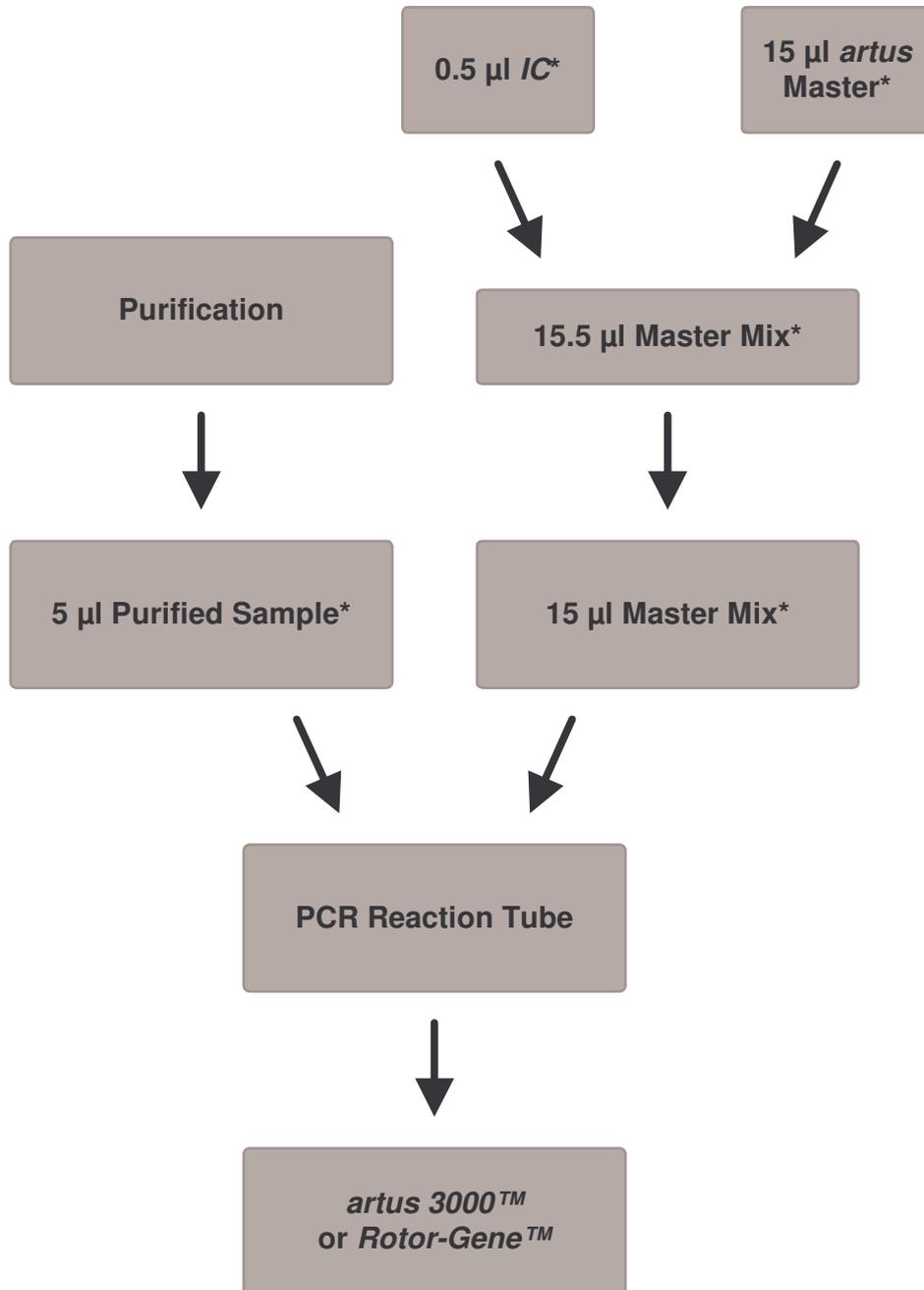


Fig. 2: Schematic workflow for the control of PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

8.6 Programming of the *artus 3000*TM or *Rotor-Gene*TM 3000

For the detection of *Plasmodium* DNA, create a temperature profile on your *artus 3000*TM or *Rotor-Gene*TM 3000 according to the following five steps (see Fig. 3 - 7).

- | | | |
|----|---|--------|
| A. | Setting of General Assay Parameters | Fig. 3 |
| B. | Initial Activation of the Hot Start Enzyme | Fig. 4 |
| C. | Amplification of the DNA | Fig. 5 |
| D. | Adjustment of the Fluorescence Channel Sensitivity | Fig. 6 |
| E. | Starting of the <i>artus 3000</i> TM or <i>Rotor-Gene</i> TM 3000 Run | Fig. 7 |

All specifications refer to the *artus 3000*TM software version 5.0.69 or *Rotor-Gene*TM software version 4.6.94. Please find further information on programming the *artus 3000*TM or *Rotor-Gene*TM 3000 in the *artus 3000*TM *Software Manual* or *Rotor-Gene*TM *Manual*, Version 4.6. In the illustrations these settings are framed in bold black.

First, enter the PCR reaction volume in the menu window *New Experiment Wizard* (see Fig. 3).

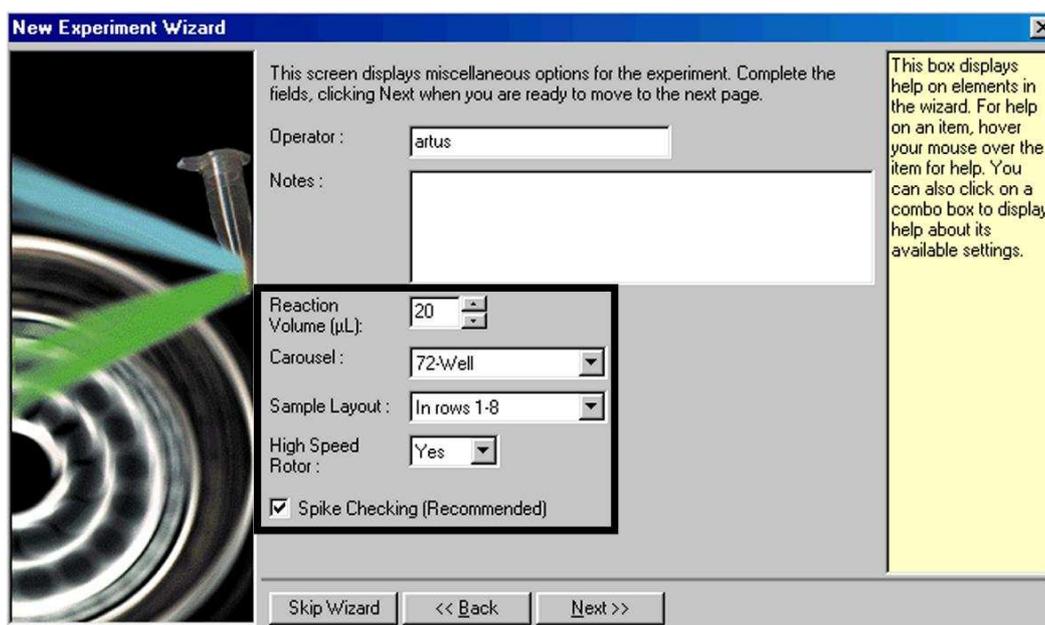


Fig. 3: Setting of General Assay Parameters.

Programming the temperature profile is done by activating the button *Edit* in the next *New Experiment Wizard* menu window (see Fig. 4 and 5).

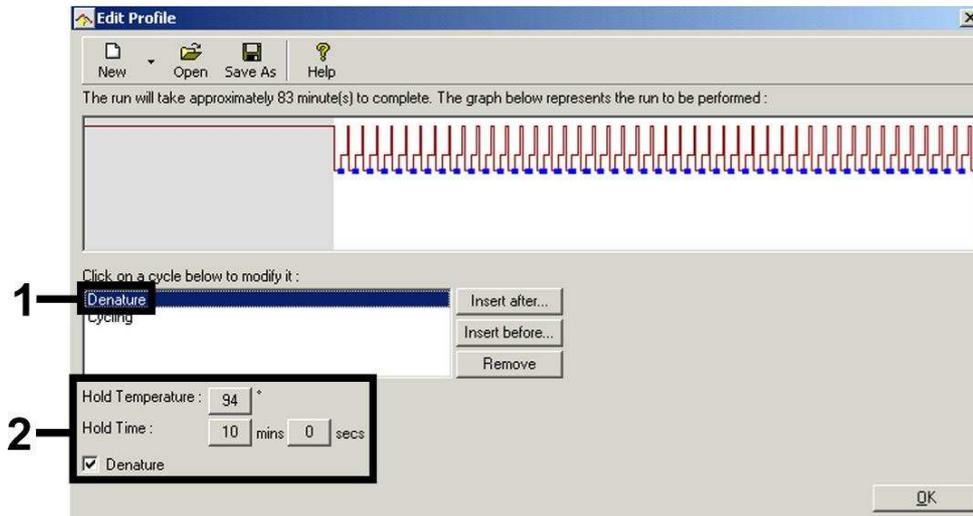


Fig. 4: Initial Activation of the Hot Start Enzyme.

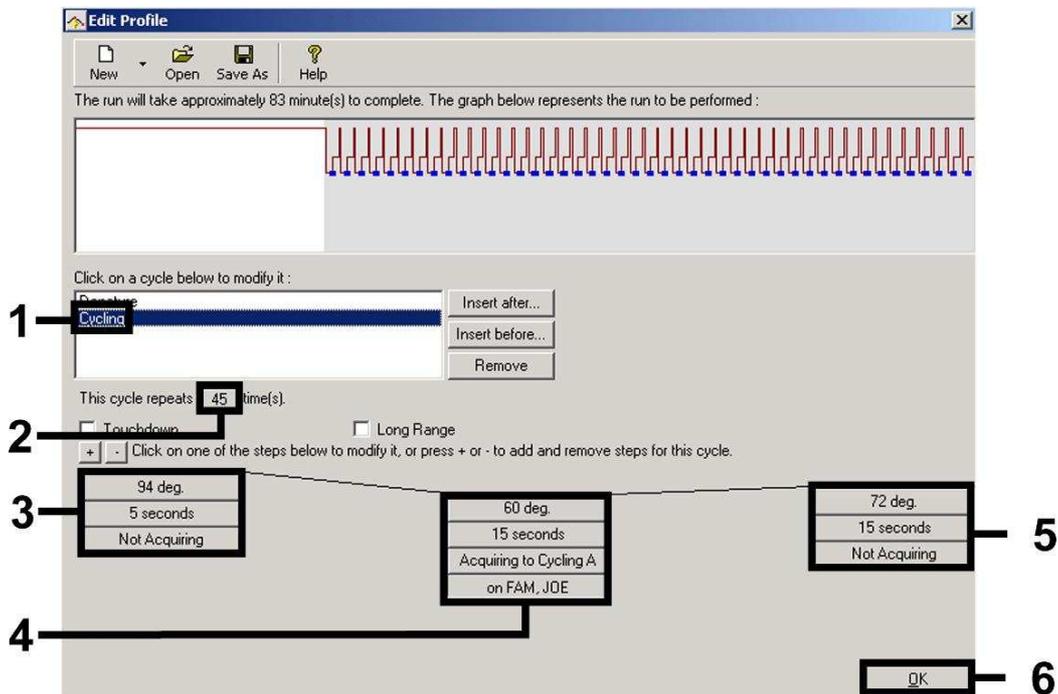


Fig. 5: Amplification of the DNA.

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain Calibration Setup* (activation in menu window *New Experiment Wizard* under *Calibrate*). Please set the calibration temperature to the annealing temperature of the amplification programme (see Fig. 6).

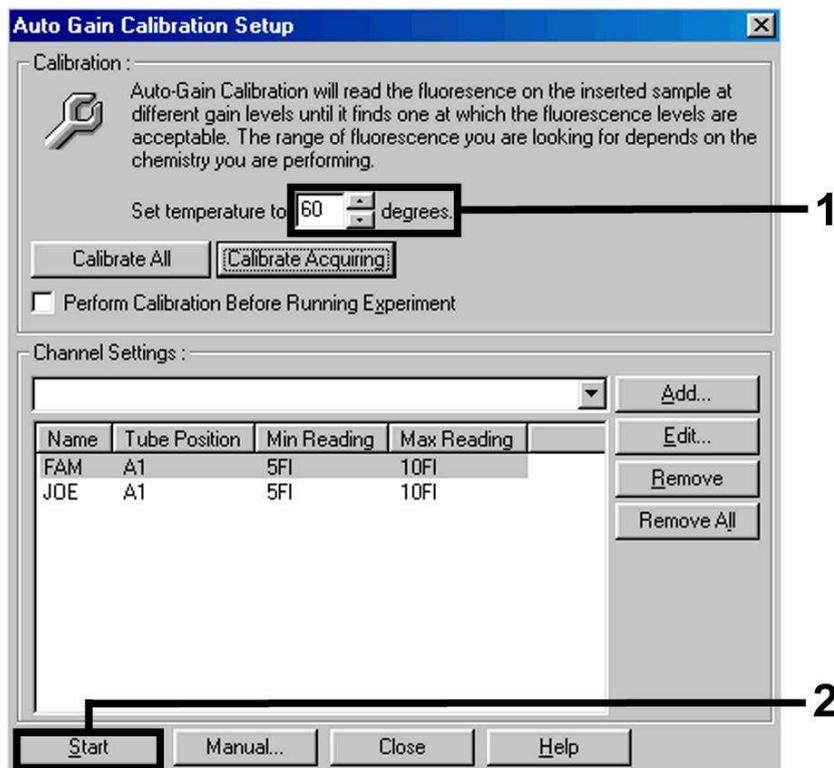


Fig. 6: Adjustment of the Fluorescence Channel Sensitivity.

The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (see Fig. 7).

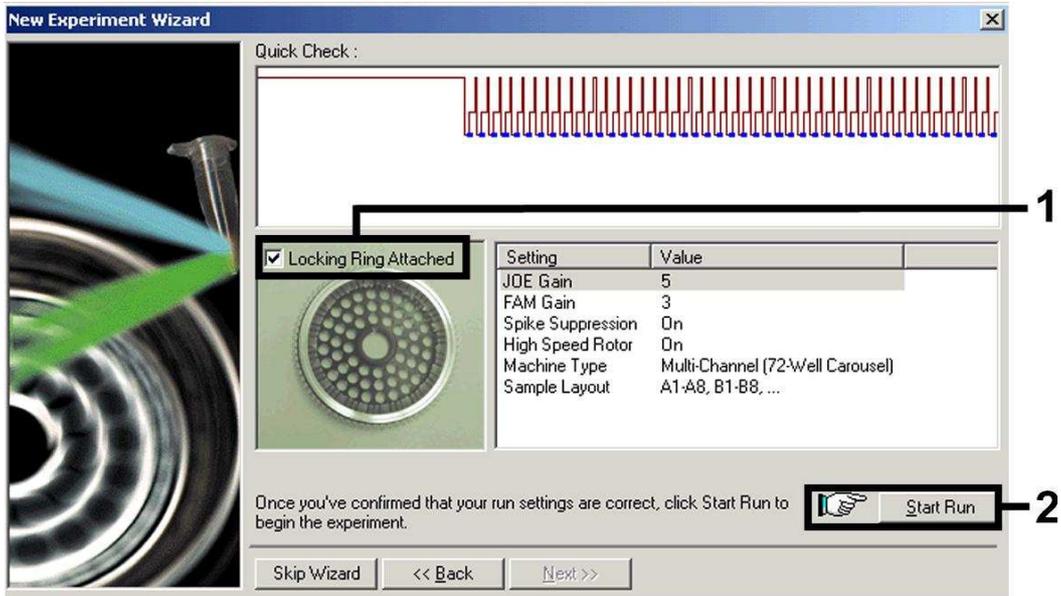


Fig. 7: Starting of the *artus 3000*[™] or *Rotor-Gene*[™] 3000 Run.

9. Data Analysis

Data analysis is performed with the *artus 3000™* or *Rotor-Gene™* software according to the manufacturer's instructions (*artus 3000™ Software Manual* or *Rotor-Gene™ Manual*, Version 4.6).

The following results are possible:

1. A signal is detected in fluorescence channel Cycling A.FAM.

The result of the analysis is positive: The sample contains *Plasmodium* DNA.

In this case, the detection of a signal in the Cycling A.JOE channel is dispensable, since high initial concentrations of *Plasmodium* DNA (positive signal in the Cycling A.FAM channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the Cycling A.JOE channel (competition).

2. In fluorescence channel Cycling A.FAM no signal is detected. At the same time, a signal from the *Internal Control* appears in the Cycling A.JOE channel.

In the sample no *Plasmodium* DNA is detectable. It can be considered negative.

In the case of a negative *Plasmodium* PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

3. No signal is detected in the Cycling A.FAM or in the Cycling A.JOE channel.

No result can be concluded.

Information regarding error sources and their solution can be found in **10. Troubleshooting.**

Examples of positive and negative PCR reactions are given in Fig. 8 and Fig. 9.

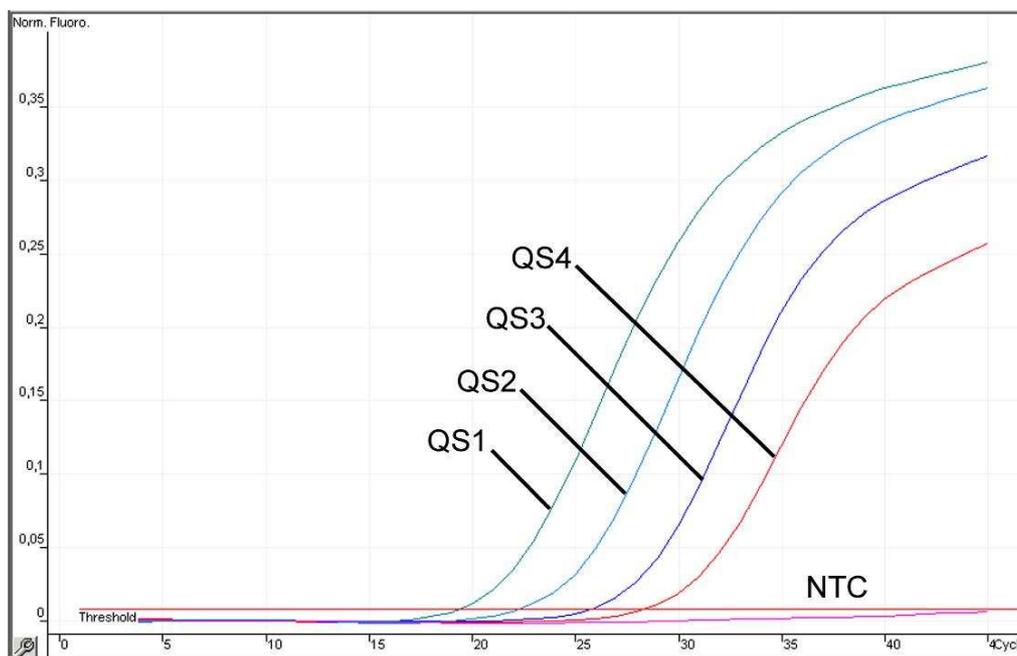


Fig. 8: Detection of the *Quantitation Standards (Malaria RG QS 1 - 4)* in fluorescence channel Cycling A.FAM. NTC: non-template control (negative control).

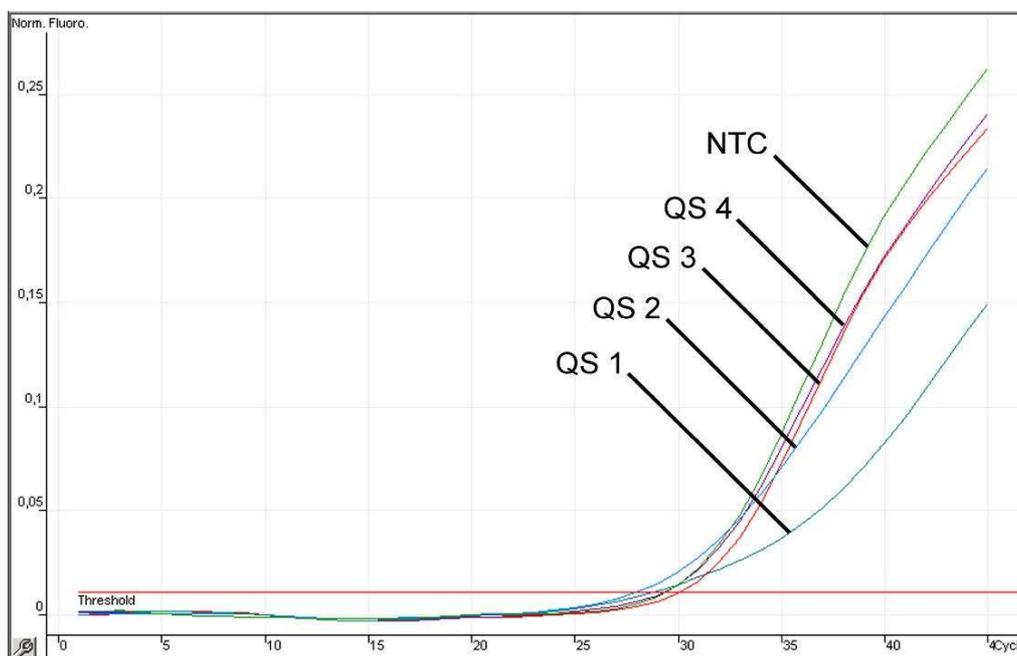


Fig. 9: Detection of the *Internal Control (IC)* in fluorescence channel Cycling A.JOE with simultaneous amplification of the *Quantitation Standards (Malaria RG QS 1 - 4)*. NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*Malaria RG QS 1 - 4*) in fluorescence channel Cycling A.FAM:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.
 - For data analysis select the fluorescence channel A.FAM for the analytical *Plasmodium* PCR and the fluorescence channel A.JOE for the *Internal Control* PCR.
- Incorrect programming of the temperature profile of the *artus 3000™* or *Rotor-Gene™ 3000*.
 - Compare the temperature profile with the protocol (see **8.6 Programming of the *artus 3000™* or *Rotor-Gene™ 3000***).
- Incorrect configuration of the PCR reaction.
 - Check your work steps by means of the pipetting scheme (see **8.5 Preparing the PCR**) and repeat the PCR, if necessary.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus Malaria RG PCR Kit* had expired.
 - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Weak or no signal of the *Internal Control* in fluorescence channel Cycling A.JOE and simultaneous absence of a signal in channel Cycling A.FAM:

- The PCR conditions do not comply with the protocol.
 - Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
 - Make sure that you use a recommended isolation method (see **8.2 DNA Isolation**) and stick closely to the manufacturer's instructions.

- Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see **8.2 DNA Isolation**).
- DNA was lost during extraction.
 - If the *Internal Control* had been added to the extraction, an absent signal of the *Internal Control* can indicate the loss of DNA during the extraction. Make sure that you use a recommended isolation method (see **8.2 DNA Isolation**) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* Malaria RG PCR Kit had expired.
 - Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

**Signals with the negative controls in fluorescence channel
Cycling A.FAM of the analytical PCR.**

- A contamination occurred during preparation of the PCR.
 - Repeat the PCR with new reagents in replicates.
 - If possible, close the PCR tubes directly after addition of the sample to be tested.
 - Strictly pipette the positive controls at last.
 - Make sure that work space and instruments are decontaminated at regular intervals.
- A contamination occurred during extraction.
 - Repeat the extraction and PCR of the sample to be tested using new reagents.
 - Make sure that work space and instruments are decontaminated at regular intervals.

If you have any further questions or if you encounter problems, please contact our Technical Service.

11. Specifications

11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* Malaria RG PCR Kit, a standard dilution series has been set up from 758 to nominal 0.24 *Plasmodium* copy equivalents*/ μl and analysed with the *artus* Malaria RG PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 10. The analytical detection limit of the *artus* Malaria RG PCR Kit is consistently 0.5 copies/ μl ($p = 0.05$). This means that there is a 95 % probability that 0.5 copies/ μl will be detected.

Probit analysis: *Plasmodium* spp. (*artus* 3000™/Rotor-Gene™ 3000)

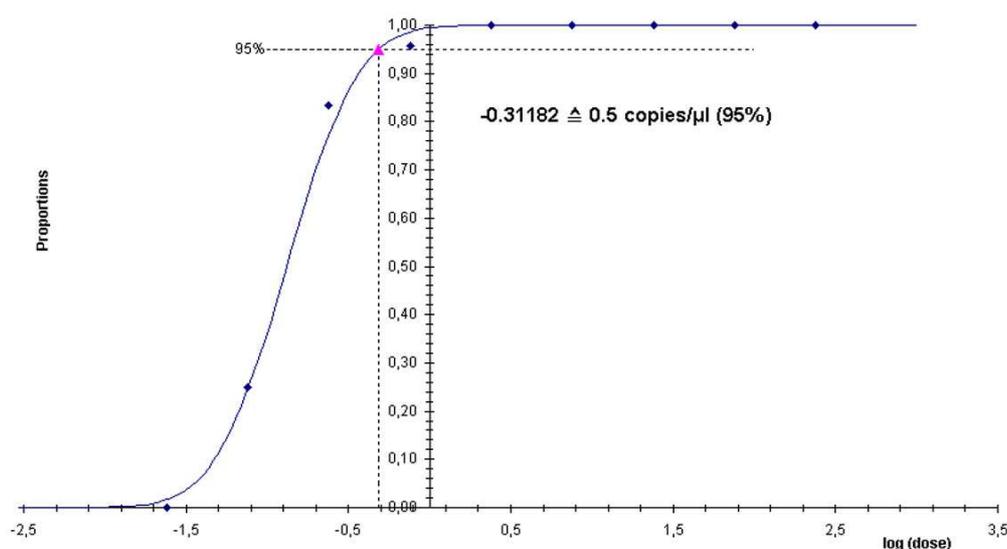


Fig. 10: Analytical sensitivity of the *artus* Malaria RG PCR Kit.

* The standard is a cloned PCR product, the concentration of which has been determined by absorption and fluorescence spectroscopy.

11.2 Specificity

The specificity of the *artus* Malaria RG PCR Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. The detectability of all relevant *Plasmodium* species has thus been ensured by a database alignment and by a PCR run on *artus 3000™* or *Rotor-Gene™ 3000* with the following *Plasmodium* species (see Table 1).

Table 1: Testing of the specificity of relevant *Plasmodium* species.

<i>Plasmodium</i> species	Source	<i>Plasmodium</i> (Cycling A.FAM)	Internal Control (Cycling A.JOE)
<i>P. falciparum</i>	ATCC [®] MRA-177	+	+
<i>P. vivax</i>	ATCC [®] MRA-178	+	+
<i>P. malariae</i>	ATCC [®] MRA-179	+	+
<i>P. ovale</i>	ATCC [®] MRA-180	+	+

[®] ATCC = American Type Culture Collection

The *artus* Malaria RG PCR Kit does also generate positive signals with other non-human pathogenic species such as *Plasmodium berghei* from mouse. However, these species are not pathogenic to humans since they are unable to exist in humans.

Moreover, the specificity was validated with 30 different *Plasmodium* negative human blood samples. These did not generate any signals with the *Plasmodium* specific primers and probes, which are included in the *Malaria RG Master*.

To determine the specificity of the *artus* Malaria RG PCR Kit the control group listed in the following table (see Table 2) has been tested for cross-reactivity. None of the tested samples has been reactive.

Table 2: Testing the specificity of the kit with potentially cross-reactive samples.

Control group	<i>Plasmodium</i> (Cycling A.FAM)	<i>Internal Control</i> (Cycling A.JOE)
<i>Trypanosoma cruzi</i>	-	+
<i>Trypanosoma brucei</i>	-	+
<i>Leishmania major</i>	-	+
<i>Leishmania brasiliensis</i>	-	+
<i>Leishmania mexicana mexicana</i>	-	+
<i>Leishmania donovani</i>	-	+
<i>Toxoplasma gondii</i>	-	+
<i>Theileria annulata</i>	-	+
<i>Babesia spec.</i>	-	+

11.3 Precision

The precision data of the *artus* Malaria RG PCR Kit allow the determination of the total variance of the assay. The total variance consists of the **intra-assay variability** (variability of multiple results of samples of the same concentration within one experiment), the **inter-assay variability** (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the **inter-batch variability** (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the *Internal Control* PCR.

Precision data of the *artus* Malaria RG PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (QS 4; 70 copies/μl). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 3). In addition, precision data for quantitative results in copies/μl were determined using the corresponding Ct values (see Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.59 % (Ct) or 12.65 % (conc.), for the

detection of the *Internal Control* 1.99 % (Ct). These values are based on the totality of all single values of the determined variabilities.

Table 3: Precision data on basis of the Ct values.

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: <i>Malaria RG QS 4</i>	0.50	0.26	1.67
Intra-assay variability: <i>Internal Control</i>	0.37	0.14	1.34
Inter-assay variability: <i>Malaria RG QS 4</i>	0.51	0.26	1.67
Inter-assay variability: <i>Internal Control</i>	0.66	0.44	2.41
Inter-batch variability: <i>Malaria RG QS 4</i>	0.42	0.17	1.39
Inter-batch variability: <i>Internal Control</i>	0.47	0.22	1.70
Total variance: <i>Malaria RG QS 4</i>	0.48	0.23	1.59
Total variance: <i>Internal Control</i>	0.55	0.30	1.99

Table 4: Precision data on basis of the quantitative results (in copies/ μ l).

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: <i>Malaria RG QS 4</i>	10.29	105.98	14.57
Inter-assay variability: <i>Malaria RG QS 4</i>	9.24	85.31	13.09
Inter-batch variability: <i>Malaria RG QS 4</i>	8.54	72.92	12.11
Total variance: <i>Malaria RG QS 4</i>	8.93	79.66	12.65

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* Malaria RG PCR Kit. 30 *Plasmodium* negative samples of blood were spiked with 1.5 copies/μl elution volume of *Plasmodium* control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the High Pure PCR Template Preparation Kit (Roche Diagnostics; see **8.28.1 DNA Isolation**) these samples were analysed with the *artus* Malaria RG PCR Kit. For all *Plasmodium* samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 *Plasmodium* negative blood samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* Malaria RG PCR Kit is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* Malaria RG PCR Kit as well as an efficiency comparison with other products. These data are obtained by performing evaluation studies (see **11.6 Diagnostic Evaluation**).

11.6 Diagnostic Evaluation

Currently, the *artus* Malaria RG PCR Kit is undergoing a series of evaluation studies.

12. Product Use Limitations

- The *artus* Malaria RG PCR Kit is for research use only. Not for use in diagnostic procedures.
- No claim or representation is intended for their use for a specific clinical use (diagnostic, prognostic, or therapeutic).
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

13. Safety information

For safety information of the *artus* Malaria RG PCR Kit, please consult the appropriate material safety data sheet (MSDS). The MSDS are available online in convenient and compact PDF format at www.qiagen.com/support/msds.aspx.

14. Quality control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Quality Management System, each lot of *artus* Malaria RG PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

15. References

Mackay IM. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004; 10 (3): 190 - 212.

16. Explanation of Symbols



Use by



Batch code



Manufacturer



Catalogue number



Material number



Handbook



Contains sufficient for <N> tests



Temperature limitation

QS

Quantitation Standard

IC

Internal Control

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